OBSERVATIONS ON THE FLUORESCENT MATERIAL IN HAIRS INFECTED BY MICROSPORON IN TINEA CAPITIS*

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The greenish fluorescence of children's hair infected by M. audouni and M. lanosum under the Wood light is now a widely known phenomenon. Very little work, however, has been done in determining the nature and source of this fluorescent material. Kinnear (1) claimed that it was the fungus itself that fluoresced. He found that if the infected hair was put into liquor potassi the hair became swollen and dissociated, and that the detached sheath of spores still fluoresced as a group. Davidson and Gregory (2), however, in observations of microtome sections of hair follicles found that the spore sheath fluoresced a faint bluish-white while the hair shaft was bright green. These authors concluded that only that part of the hair which was actually penetrated by fungal hyphae was fluorescent, and that this fluorescence was due to some change in the hair substance, perhaps a product of keratin hydrolysis. They extracted the fluorescent substance by heating ether-treated dried hair in water. The water extract was found to fluoresce with the characteristic green color. In a preliminary chemical examination, apparently not completed, they found that the green substance contained nitrogen, an aldehyde group, and a phenolic ring.

Benedek (3) found that presence of spores seemed to be necessary for fluorescence. In some long standing infections with microsporon he noted that the typical greenish fluorescence was absent in hairs which had no spore sheath. In these hairs only endothrix mycelia were found, yet on culture microsporon audouini grew out.

It is well known that the microsporon fungi in culture on Saboraud's media do not show the characteristic green fluorescence seen on the infected scalp. Lewis (4) found that M. audouini fluoresced grayish-brown, while M. lanosum showed a tan centre with the rest of the colony being bluish-lavender after twenty-one days' growth. Using a special liquid media containing urea and cane sugar, Mallinckrodt-Haupt and Carrie (5) obtained a greenish fluorescence of the filtrate which became bluish on the addition of acid and green again on the addition of alkali.

EXPERIMENTAL

A. Extraction of the Green Fluorescent Substance from Infected Hair

The green fluorescent substance was found to be readily soluble in hot water and was easily extracted by the method of Davidson and Gregory (2). It was

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also found that a concentrated solution of sodium bromide (2N) readily dissolved out the fluorescent material in the cold.

Method I. A small amount of infected epilated hair was first allowed to soak in ether for 24–48 hours, dried, a few cc. of water added and then heated in a water bath for 15–30 minutes. Examination of the water filtrate showed the characteristic greenish fluorescence under the Wood light. The extracted hair lost a great deal of its fluorescence.

Method II. A small amount of infected epilated hair was allowed to soak in 5–10 cc. of a 2N sodium bromide solution for 30 minutes at room temperature. The filtrate showed the characteristic greenish fluorescence.

B. Fluorescent Color Changes at Various ph's

The color of the fluorescence was found to change with the ph of the solution (table 1). The water extract was green in acid at ph 5.5 and blue in alkaline solution at ph 8.0. In the sodium bromide extract a much wider difference in

TABLE 1

Effect of ph on the Color of Fluorescence

FLUORESCENCE	pH
Water Extract	
Green	5.5
Blue-green	6.0–7.5
Blue	8.0
Sodium Bromide Extract	
Green	9.0
Blue-green	8.0–2.5
Blue	2.0

ph had to be obtained before a color change was noted; and, then, it was opposite to that in water extracts, namely, green in alkaline at ph 9.0 and blue in acid at ph 2.0.

C. Quenching of Fluorescence

Various compounds are known to have the ability to suppress fluorescence, the halogens being especially prominent among these. The ability of these ions to quench fluorescence corresponds approximately with the salting out activity of the ions arranged according to the so-called Hofmeister series as follows: $SCN^- > I^- > Br^- > Cl^-$ (6). Antioxidants such as CN^- also quench fluorescence. The effect of various salt solutions on the fluorescence of tinea infected hairs is shown in table 2. The CN^-, SCN^- and I^- ions quenched the fluorescence, whereas Br^- and Cl^- ions had no such action. Table 3 shows the effect of adding a few drops of 2N KI to a few cc. of the fluorescent heat extracted water solution, and sodium bromide solution of infected hair. Complete quenching occurred only in the acid solutions of both extracts.

The fluorescence can also be easily quenched *in vivo* by adding 10 or 20% potassium iodide to a fungicidal ointment and massaging it into the scalp. After only one such application, the fluorescence was practically entirely gone by the following day. If hair in the infected area, however, was epilated the green fluorescence could still be noted at the roots. When the potassium iodide ointment was washed out of the scalp the fluorescence rapidly returned within 12 to 24 hours to the entire infected area. The absence of fluorescence did not influence the viability of the fungus. Thus, it may happen that if ointments are used containing iodides previous to examination, the Wood light may fail to discover the infection.

TABLE 2

Effect of neutral salt solutions on fluorescence of infected hair

SOLUTION	FLUORESCENCE
0.1M KCN	Dimmed in 20 min.
2M KCNS	Dimmed in 30 min.
2M KI	Dimmed in 45 min.
2M KBr	No effect
2M KCl	No effect
2M $CaCl_2$	No effect

TABLE 3

Effect of 2N KI on fluorescent material of infected hair

EXTRACT	pH	EFFECT
Water	11.0	no quenching
Water	6.0	moderate quenching
Water	2.0	complete quenching
NaBr	11.0	no quenching
NaBr	10.0	moderate quenching
NaBr	2.0	complete quenching

D. Microscopic Findings in Naturally Infected Hair

It has already been stated that some controversy exists as to the source of fluorescence in ringworm of the scalp due to microsporon. Using chlorallactophenol as a swelling agent for the infected hair the typical green fluorescence could very well be seen under the microscope using the Wood lamp as a light source. By noting the areas of fluorescence and then viewing the same areas with ordinary light it was seen that the fluorescence appeared to be diffusely present both in the spore sheaths and in the hair shaft even where there was no surrounding sheath of spores, or visible mycelia within the hair. Cross sections of hair (fig. 1) also revealed fluorescence both in the spore sheath and within the hair shaft, the latter usually showing the most intense fluorescence.

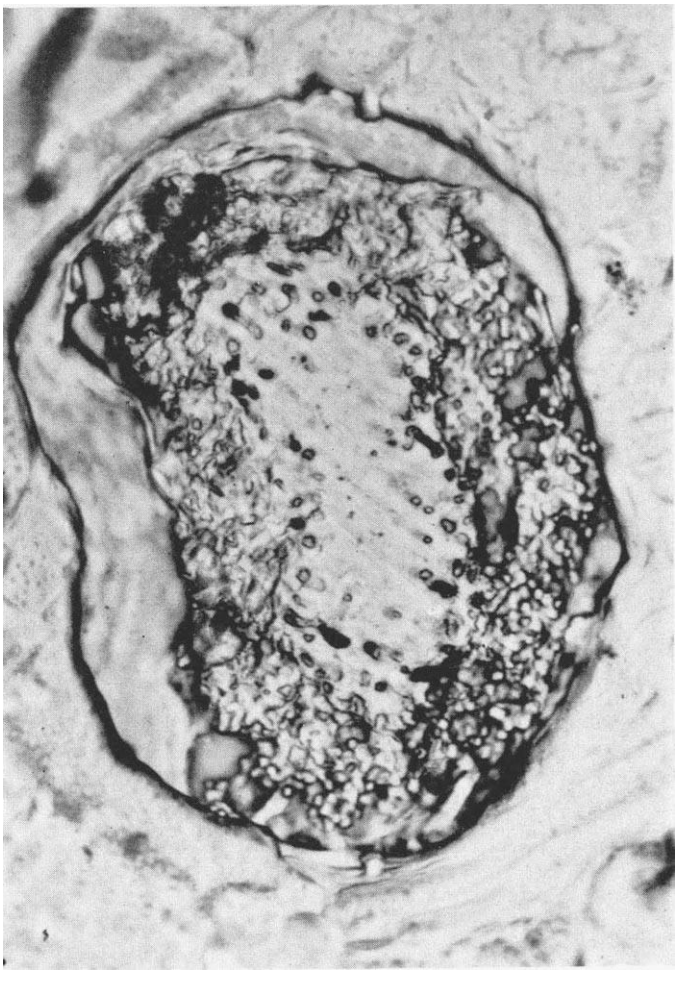

FIG. 1. Cross section of epilated microsporon infected hair showing spore sheath

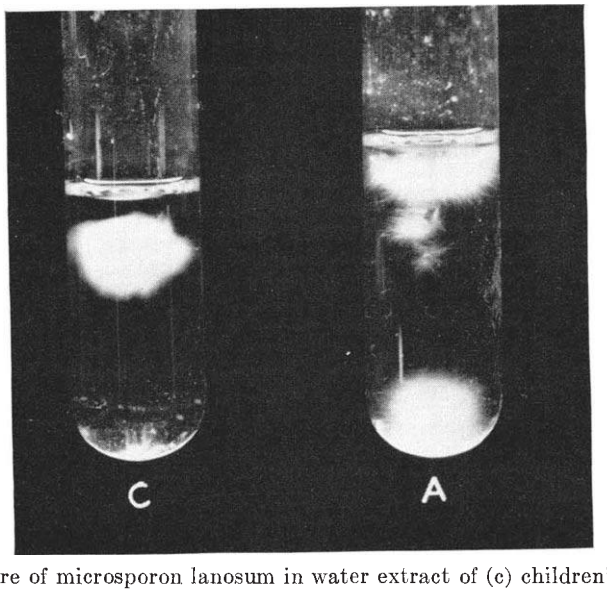

FIG. 2. Culture of microsporon lanosum in water extract of (c) children's hair and (a) adult hair.

E. Behavior of Artificially Infected Hair

In epilated hair artificially infected by M. audouini and M. lanosum no fluorescence could be obtained, although on microscopic examination a rich growth of mycelial elements could be seen about the hairs. No microspores formed in these artificial cultures using only adult or children's hair plus a small amount of water for a culture medium.

F. Behavior of Cultures Grown in Hair Extracts

Both M. audouini and M. lanosum could also be grown in water extracts of either children's or adults' hair. These extracts were made by allowing the hair to soak in triple distilled water at room temperature for several days or by gently heating the hair in water for a few minutes on a water bath. Definite fluffy growth of the fungi was obtained (fig. 2) but no real green fluorescence changing with change of ph was found in the filtrate.

COMMENT

The fluorescent compound from microsporon infected hair belongs to the group of so-called fluorescent indicator substances that change color at different ph's. There is no common chemical structure to these compounds, and no conclusions can be drawn as to the structure of the substance from this phenomenon. The reversal of color change in the sodium bromide solution is in keeping with the fact that both absorption and fluorescent spectra can be shifted to different wave lengths according to the solvent employed. The shift may be connected with the dipole character of the solvent (6).

Fluorescence is present both in the spore-containing hair sheath and in the hair shaft of naturally infected hair. Whereas in such hair microspores are formed in sheaths and mycelia are present in the shaft, such a process cannot be elicited by *in vitro* infection of hair. Fluorescence in hair arises only under conditions of the development of the fungus *in vivo*. The fluorescent material may be a secretion or excretion product of fungus metabolism, but whether hair proteins are also involved in its production cannot be stated at the present time.

SUMMARY

1. The green fluorescent material of microsporon infected hair can be extracted by hot water or cold 2N sodium bromide solution.

2. The fluorescence changes color at various ph's and is due to the presence of a fluorescent indicator substance. Various ions quench the fluorescence without influencing the viability of the fungus. Fluorescence is not present in *in vitro* infected hairs.

3. Fluorescence is present both in the spore-containing hair sheath and in the mycelium-containing hair shaft of naturally infected hair.

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